consideration as being drawn to a non-elected group.

Claims 1-13, 22 and 23 stand rejected.

Rejection Under 35 U.S.C. §103

Claims 1-6 and 8-12 stand rejected under 35 U.S.C.§103 as obvious over Markowicz et al. in view of Jakoby et al. Claims 7 and 13 stand rejected under 35 U.S.C. §103 as obvious over Markowicz et al. in view of Jakoby et al. and in further view of Koch et al. Both of these grounds of rejection are premised on the Examiner's continued reliance on Markowicz et al. as teaching "that dendritic precursors when exposed to GM-CSF will proliferate into dendritic cells... ." The Examiner "disagrees" with Applicants' assertions that Markowicz et al. fails to provide evidence of proliferating dendritic cell precursors or their response to GM-CSF.

The Examiner states that "the teachings of Markowicz et al. and the motivation to apply the teachings was set forth many Office Actions ago."

Applicants request reconsideration of their previous submissions and again emphasize the failure of Markowicz et al. to identify the proliferating cells which they report may be present in their cultures. As applicants previously explained, Markowicz et al. states

> As shown in Fig. 4. the number of differentiated (branched DC) increased as the concentration of GM-CSF in the culture increased. At any given concentration of the cytokine, however, the total number of viable cells as well as the number of branched cells per well remained stable over time suggesting that GM-CSF does not cause DC to divide and proliferate.

Markowicz et al., page 958, emphases added. Markowicz et al. unequivocally therefore

teaches away from concluding that the cultures have proliferating dendritic cell precursors. Moreover, the data presented in Fig. 4 of Markowicz et al. also indicates that the dendritic cells are about ten percent (10%) of the total cell population per well (10,000 - 20,000 total viable cells per culture period per well and about 1000 branched (dendritic) cells per well). Markowicz, et al. state that "Branched DC typically comprised 10-40% of the total number of viable cells in cultures supplemented with GM-CSF." Page 959. Thus, if some dendritic cells are in fact dying, the number of dendritic cells may be maintained at a stable number by another form of non-proliferating cell maturing into a branched dendritic cell, while other non-dendritic cells proliferate keeping the total cell number constant. Markowicz et al. states that "The remaining cells consisted primarily of unbranched DC (DR+, Leu M3- cells capable of differentiating into branched adherent cells when transferred), and smaller numbers of macrophages and lymphoid cells." Id. at 959. Such unbranched dendritic cells may replace branched dendritic cells which die and the population may be kept constant by proliferating macrophages or lymphoid cells. Markowicz et al. reports the proliferation of lymphocytes when IL-2 was added to unfractionated cultures demonstrating the capacity of cells to proliferate.

Consistent with this analysis is the lack of increase in dendritic cell number over time from day 11 to day 24 as shown in Figure 4. Clearly, if a population of dendritic cells were proliferating as claimed by Applicants this number should increase over time. This is in stark contrast to Applicants' invention which clearly demonstrates expansion of dendritic cell cultures from proliferating dendritic cell precursors. As stated by Applicants:

> In summary, from a starting blood mononuclear culture of 1.5×10^6 cells, where dendritic cells were difficult to detect, we on average obtained 5-10 subcultures each with at least $3\text{-}10 \times 10^4$ released dendritic cells at 3 weeks, as well as many aggregates capable of further proliferation.

Page 52, line 33 – page 53, line 3. Applicants' invention is therefore clearly not made obvious by Markowicz et al. as Markowicz et al. provides no evidence of proliferating dendritic cell precursors.

Further evidence of the lack of a teaching or suggestion to obtain proliferating dendritic cells as claimed by Applicants is the failure of Markowicz et al. to appreciate the significance of the difference in antigen expression of proliferating versus non-proliferating dendritic cell precursors. Markowicz et al. report that contaminating macrophages are separated from dendritic cells by panning on human IgG-coated Petri dishes. Page 956. In contrast, Applicants' invention encompasses removing non-dendritic cell precursors which may in fact include some dendritic cells. As stated in the application:

> The starting material for the method of producing dendritic cell precursors and mature dendritic cells is a tissue source comprising dendritic cell precursors which precursor cells are capable of proliferating and maturing in vitro into dendritic cells when treated according to the method of the invention. Such precursor cells are nonadherent and typically **do not label with mAb markers found on dendritic cells**....

Page 24, lines 16-23. The application further states:

> According to a method of the invention, the tissue source may be treated prior to culturing to enrich the proportion of dendritic cell precursor cells relative to other cell types. Such pretreatment may also remove cells which may compete with the proliferation of dendritic cell precursor cells or inhibit their proliferation or survival.

Page 25, lines 18-23. The distinction between selecting for precursor cells rather than mature dendritic cells is further emphasized by Applicants' teaching that both mature dendritic cells and macrophages may be killed to increase the proportion of dendritic cell precursors which express different antigens than mature dendritic cells.

> In addition, Ia positive cells, i.e. B cells and macrophages preferably are killed by culturing the cells in the presence of a mixture of anti Ia-antibodies, preferably monoclonal antibodies, and complement. Mature dendritic cells which are also present in bone marrow are also killed when the cells from the bone marrow are cultured in the presence of anti Ia-antibodies, however, these mature dendritic cells occur in such low quantities in the blood and bone marrow and possess such distinct antigenic markers from dendritic cell precursors that killing of these mature dendritic cells will not significantly effect the proliferation and yield of dendritic cell precursors.

Page 27, lines 15-26. Markowicz et al. therefore clearly teaches away from applicants' invention.

In view of the above, Applicants respectfully request reconsideration and removal of all remaining grounds of rejection and allowance of the pending claims.

Respectfully submitted,

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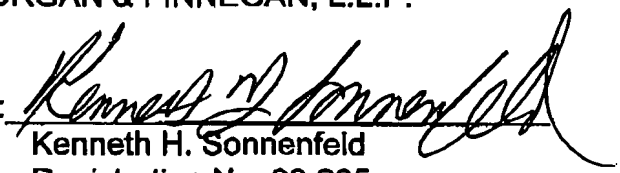
Dated: August 4, 1998

By: 
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